Streptococcal Pyrogenic Exotoxin A Release, Distribution, and Role in a Murine Model of Fasciitis and Multiorgan Failure Due to *Streptococcus pyogenes*

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The role of streptococcal pyrogenic exotoxin A (SPEA) was evaluated in a murine model of fasciitis and multiorgan failure due to a toxigenic strain of *Streptococcus pyogenes*. Increased serum levels of SPEA at 15 and 21 h were associated with a survival time of <24 h. Levels of SPEA correlated with interleukin-6 levels. Immunostaining showed SPEA localized to renal and hepatic cells. Neutralizing rabbit antibody to SPEA was administered to mice challenged with *S. pyogenes*, but no effect on survival was observed. Vaccination of mice with recombinant SPEA enhanced mortality due to streptococcal infection, despite the development of neutralizing immunity to the toxin prior to infection. Hence, SPEA is produced systemically during *S. pyogenes* soft-tissue infection, and increased levels are associated with reduced survival. In this model, however, SPEA did not appear to play a dominant role in pathogenesis; passive immunization against SPEA was not protective, and active immunization enhanced mortality.

*Streptococcus pyogenes* (group A streptococcus) is the causative agent of a wide spectrum of disease, ranging from streptococcal pharyngitis and impetigo to life-threatening necrotizing fasciitis and the recently recognized streptococcal toxic-shock syndrome (STSS) [1]. Several streptococcal virulence factors have been implicated in the pathogenesis of necrotizing fasciitis and STSS, such as protease production, M1/M3 serotype, and expression of superantigenic toxins [2-4].

Accumulating epidemiologic evidence suggests that the production of superantigens, such as streptococcal pyrogenic exotoxin A (SPEA), by some strains of *S. pyogenes* may play an important pathogenic role in STSS [5]. SPEA at concentrations as low as 10 ng/mL can, in the presence of antigen-presenting cells, stimulate human variable β chain (Vβ) 2+ and Vβ12+ T cells to proliferate in vitro [6]. It is hypothesized that superantigen-producing strains of *S. pyogenes* associated with STSS cause superantigen-mediated effects in vivo, resulting in some of the features of this disease, such as rash, hypotension, acute respiratory distress syndrome, and lymphocyte depletion [7, 8]. However, it is unknown whether superantigens such as SPEA are produced systemically during soft-tissue infection with *S. pyogenes* and whether SPEA circulates at levels sufficient to cause superantigen-mediated effects.

The purpose of this study was to evaluate comprehensively the pathogenic role of SPEA in a murine model of lethal streptococcal necrotizing fasciitis. First, we did a detailed examination of the histologic features of the model, with special reference to aspects that might reflect evidence of superantigenicity in vivo. Second, we evaluated the production and distribution of SPEA in vivo and related these findings to histologic findings, systemic inflammatory response, and survival. Finally, to directly address the possible role of SPEA in causing tissue injury and death in this model, we compared the murine response to recombinant (r) SPEA with the response to infection with SPEA-producing streptococci and evaluated the effects of both active and passive vaccination on survival.

**Materials and Methods**

**Mice.** Male, 6- to 8-week-old CD1 mice (Charles River, Margate, UK) weighing 22–25 g were used in all experiments.

**Bacteria and media.** A scarlet fever serotype M1T1 *S. pyogenes* isolate, H250, shown by immunodiffusion to produce SPEA but not streptococcal pyrogenic exotoxin C (Streptococcal Reference Laboratory, Central Public Health Laboratory, Colindale, UK) was used in all experiments involving live streptococcal septis. This isolate produces 2–3 µg/mL SPEA in rich medium as measured by ELISA. For intramuscular (im) challenge, a fresh colony was inoculated into Todd Hewitt broth (Oxoid, Basingstoke, UK) and incubated for 8 h at 37°C. Bacteria were then harvested by centrifugation and resuspended in pyrogen-free saline; bacterial density was determined by measuring the optical density at 325 nm (A325). The suspension was then diluted to 2 × 10^10 cfu/mL with pyrogen-free saline, using standard growth curves to relate measured A325 to bacterial concentration. For quantification of bacteremia, samples were plated onto blood agar plates and incubated overnight at 37°C. A type strain of *Staphylococcus epidermidis* (NCTC 11047) was cultured and prepared in the same way.

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All experiments involving the use of animals were conducted in accordance with local ethical guidelines and Home Office regulations.

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Animal model of infection and experimental design. A modification of the Selbie thigh lesion test [9] was used to initiate infection in 14 mice. Bacteria were inoculated into the right thigh by im injection of 0.1 mL of H250 bacterial suspension (LD50 = 2 \times 10^9 cfu/mouse). At 25 h, 4 animals were sacrificed and tissues (thigh muscle, spleen, liver, kidney, small bowel) were formalin-fixed for histologic examination. Samples were also fixed in 1% paraformaldehyde for immunohistochemistry. Blood was obtained at 5, 15, and 25 h after bacterial injection from tail veins and was serially diluted in sterile heparinized saline for quantification of bacteremia. Control animals received 0.1 mL of heat-killed H250 (70°C for 15 min), 0.1 mL (2 \times 10^9 cfu) of S. epidermidis, or 0.1 mL of pyrogen-free saline.

Immunohistochemical studies. Paraformaldehyde-fixed tissues were incubated with postimmune rabbit anti-rSPEA and stained with the avidin–biotinylated peroxidase complex method. Tissues were incubated successively with rabbit anti-rSPEA, biotinylated goat anti-rabbit IgG, and avidin-biotin-peroxidase (Vector Laboratories, Burlingame, CA) before development using diaminobenzidine with nickel enhancement [10]. Staining specificity was ensured by determining the absence of staining with preimmune serum and demonstrating competition for antibody in the presence of excess rSPEA.

Quantitative evaluation of cytokine and SPEA production in vivo. After infection with an LD50 of H250, 10 animals were studied sequentially, and blood samples were taken from tail veins at 15, 21, and 39 h. Serum was obtained from these samples by centrifugation at 10,000 g for 5 min and stored at −20°C until assayed. Blood samples were also taken from control saline-treated animals and serum obtained.

Cytokine measurements. Interleukin-6 (IL-6) levels in serum were determined by ELISA (AMS Biotechnology, Witten, UK). Tumor necrosis factor-α (TNF-α) was also measured by ELISA (Genzyme, Boston). Serum from uninfected CD1 mice at time 0 was present in the diluent used for standard concentrations of cytokines and provided a negative control for each ELISA.

rSPEA. rSPEA was expressed using the pET19b vector and His-tag purification system (Novagen, Madison, WI) according to manufacturer’s instructions. Briefly, a 700-bp BamHI product, corresponding to the published sequence for SPEA [11], was amplified from H250 bacterial DNA using the polymerase chain reaction (PCR). Primers were derived from nucleotides 1–18 and 690–708 of the mature protein sequence 5′-GGCGGATCCGCAACAAGACCAAAAGACCCCGATCACA and 3′-GGCGGATCCGACTAGTAAAAAGGTTG- CCAA. The product was cloned into pET19b and sequenced prior to transformation of BL21-DE3 Escherichia coli with the construct. Bacteria were then induced to express rSPEA by addition of isopropyl β-thiogalactopyranoside (Stratagene, La Jolla, CA), and the protein was purified from bacterial lysate on a nickel affinity-purification column.

A single 29-kDa protein was purified, corresponding to the predicted size of SPEA (26 kDa) plus the 10 histidine residue tag. Routinely, 15–18 mg of rSPEA was obtained by 50-cL culture. Contaminating endotoxin was minimized by washing the purification column with 50 vol of pyrogen-free column buffer before elution of rSPEA. Endotoxin levels of 50–100 pg/μg of SPEA were measured by limulus assay (Quadrathec, Epsom, UK). rSPEA was mitogenic to cultured human mononuclear cells at a concentration of 30 ng/mL and to cultured CD1 murine splenocytes at 1 μg/mL. The activity of rSPEA in mitogenesis and cytokine assays mirrored that of native SPEA from S. pyogenes (Toxin Technology, Sarasota, FL).

Neutralizing rabbit polyclonal antibody to SPEA. Purified rSPEA (100 μg) mixed with Freund’s complete adjuvant was injected im into 2 half-lop rabbits. Five subsequent immunizations with 10 μg of rSPEA mixed with Freund’s incomplete adjuvant (Sigma, Poole, UK) were given at 2-week intervals. Specific reactivity with rSPEA (29 kDa) and H250 supernatant (26 kDa) was present in postimmune but not preimmune rabbit serum, as assessed by Western immunoblot. Anti-rSPEA antibody was affinity-purified by passing postimmune serum over a cyanogen bromide–activated sepharose column (Pharmacia Biotech, Uppsala, Sweden) to which rSPEA was bound and eluting the antibody (according to the manufacturer’s handbook), prior to dialysis into pyrogen-free saline. Purified anti-rSPEA (400 μg/mL) completely neutralized the mitogenic effects of 600 ng/mL rSPEA in human mononuclear cell culture.

Measurement of SPEA in serum. SPEA in serum was measured by ELISA. Plates were coated with affinity-purified anti-rSPEA and samples loaded at 1/10 dilution. Biotinylated anti-rSPEA was used to detect bound SPEA, and the ELISA was developed with a streptavidin–horseradish peroxidase conjugate (Serotec, Oxford, UK). Serial dilutions of rSPEA in 10% normal mouse serum (Sigma) were used as standard concentrations. The ELISA was sensitive to 12 ng/mL and did not cross-react with serum constituents from noninfected mice nor from animals with shock due to gram-negative E. coli.

In vivo administration of rSPEA. To assess the effect of administering rSPEA alone, 200 μg in 0.4 mL of sterile pyrogen-free saline was given by intravenous (iv) tail-vein or intraperitoneal (ip) injection to 6 mice in separate experiments. Control animals received saline only. Animals were monitored for signs of systemic toxicity, and 2 animals in each group were sacrificed at 25 h for histologic examination of tissues.

Passive immunization with rabbit anti-rSPEA. To assess the role played by SPEA in the sepsis model, 4 groups of CD1 mice received an LD50 of H250 im. In 1 group, 800 μg of neutralizing rabbit polyclonal anti-rSPEA in 0.5 mL of sterile saline was given iv at 0 and 10 h. As controls, 1 group received 800 μg of purified rabbit IgG, 1 group received 800 μg of pooled human intravenous immunoglobulin (IVIG; Sigma), and 1 group received only sterile saline at the same time points. Mortality was monitored over 72 h. Tail-vein blood samples were taken at 12 h from 5 animals in each group to measure SPEA levels in serum.

Active immunization with rSPEA. Twelve mice were immunized ip with 100 μg of purified rSPEA mixed with Freund’s complete adjuvant, followed by 4 further immunizations with 10 μg of rSPEA mixed with Freund’s incomplete adjuvant over an 8-week period. Controls received immunizations with bovine serum albumin (Sigma) according to the same protocol or no immunization. Antibody to rSPEA was detected by ELISA in the serum of immunized mice at a titer of 10^8 but was not detected in the serum of nonimmunized mice. Serum from rSPEA-immunized mice neutralized the mitogenic effects of 600 ng/mL rSPEA in human mononuclear cell culture at a dilution titer of 10^-1, whereas sera from controls had no such effect.

Five mice randomly chosen from each group were weighed before infection. Mice were then infected with 10^9 cfu of H250.
In the spleen, marked structural disorganization of the architecture was apparent, and tissue sections were pale compared with all 3 control groups. Large gaps in follicles of the white pulp, loss of follicular margins, and engulfed apoptotic cells were strongly suggestive of lymphoid depletion (figure 2).

Extensive fatty change was seen throughout the liver in H250-infected animals. A striking number of apoptotic hepatocytes displayed clumping of chromatin and dense pink cytoplasm. These features were not seen in control animals. Bacterial colonies were not seen within the hepatic parenchyma.

In the small bowel, a large number of cells from intestinal crypts had been shed into the lumen in infected animals. Cells at crypt bases were necrotic without evidence of normal mitotic activity compared with all controls. Remarkably, inflammatory changes and hemorrhage were not seen.

In the kidney, changes of acute tubular necrosis were seen in H250-infected and S. pyogenes-infected animals. No such changes were seen following injection of heat-killed H250 or saline.

Results

Animal model. By 8 h after im injection of an LD₉₀ of H250, mice developed visible swelling and erythema in the infected thigh, causing altered gait. After 15 h, there was evidence of systemic illness (lethargy, ruffled fur, conjunctivitis), with extension of erythema from the thigh to the foot, groin, and tail base in some animals. No such changes were seen following injection of saline, S. epidermidis, or heat-killed H250. Deaths in the live H250–infected group occurred between 24 and 48 h after infection (figure 1). Bacteremia was not detectable 5 h after im injection of bacteria but was present in 80% of animals at 15 and 25 h (10⁷–10⁹ cfu/mL of blood). Group A streptococci were the only organisms isolated from blood cultures.

Histologic findings. An intense inflammatory infiltrate of degenerate neutrophils was seen throughout the connective tissues of the thigh, with fibrin deposition and occasional colonies of gram-positive coci. Muscle fibers adjacent to inflamed fascia were necrotic, although fibers distant from inflamed connective tissue appeared normal. These changes were not seen in any of the 3 control groups.

Figure 1. Kaplan-Meier survival plot for mice (n = 10, each group) following intramuscular inoculation of 2 x 10⁹ cfu of H250 S. pyogenes.

Figure 2. Hematoxylin-eosin–stained section of spleen from (A) H250 S. pyogenes–infected mouse and (B) saline control.
**SPEA levels in serum.** In early experiments using this model, SPEA was not detectable in serum at 5 h. In the 10 sequentially studied mice, serum SPEA reached 3.05 ± 1.7 μg/mL (mean ± SD) at 15 h, rising to 3.66 ± 2.09 μg/mL at 21 h. There was a marked variation in serum SPEA levels between individual animals. Animals that survived <24 h had significantly higher levels of SPEA at 15 h (P = .009) and 21 h (P = .02) than those surviving longer (figure 3). Considering separately the animals surviving >24 h, the level of SPEA was 2.1 ± 0.8 μg/mL at 21 h and 3.4 ± 2.6 μg/mL at 39 h, although SPEA levels rose in only 2 of 5 animals. SPEA was not detected in the serum of noninfected control animals.

**Cytokine measurements.** IL-6 levels in all 10 sequentially studied animals were elevated at 15 h (6.58 ± 4.25 ng/mL, mean ± SD) and rose to 36.5 ± 65.9 ng/mL at 21 h. Levels of IL-6 at 21 h strongly correlated with SPEA levels at the same time point (r = .845, P < .01). Higher levels of IL-6 at 21 h were associated with survival times of <24 h (P = .01). Animals surviving >24 h had a later rise in serum IL-6, from 5.05 ± 4.3 ng/mL at 21 h to 29.96 ± 40.0 ng/mL at 39 h. IL-6 was not detected in the serum of noninfected mice.

TNF-α was not detected in any mouse at 15 h and in only 1 mouse at 21 h (309 pg/mL) following H250 infection. The same mouse had its highest levels of IL-6 and SPEA at this time point. Bioactive TNF was not detected in any mouse by L929 assay, and it was therefore not feasible to test for lymphotoxin.

**Immunohistochemical staining of tissues for SPEA.** Densely stained SPEA was detected within the cytoplasm of renal tubular cells (figure 4A). No SPEA staining was seen in infected thigh muscle, where bacterial colonies were prevalent, nor in the spleen. In the liver, SPEA was patchily distributed in hepatocytes and Kupffer cells (figure 4B, C). Staining was also evident in macrophages within the myocardium.

**Direct administration of rSPEA.** No systemic effect was observed after iv or ip administration of 200 μg of rSPEA, and no mice died. Levels of rSPEA in the serum of ip-injected mice reached 5–10 μg/mL by 5 h after injection but were later undetectable by ELISA. Observations on histologic examination of tissues from 2 mice each that received iv or ip rSPEA did not differ from those of controls that had received saline and showed no similarity to histologic appearances seen in the H250 sepsis model.

**Effect of neutralizing anti-rSPEA antibody in H250 sepsis.** Administration of anti-rSPEA antibody did not significantly alter survival following H250 im challenge compared with untreated controls. Human IVIG also did not confer a significant protection in this model (figure 5). Levels of SPEA at 12 h, as measured by ELISA, were reduced by anti-rSPEA (mean ± SD, 444.7 ± 168.2 ng/mL) but not to levels significantly below those in saline-treated animals (566.1 ± 105.7 ng/mL). Levels of SPEA in human IVIG–treated mice were also lower than levels in saline-treated controls (494.12 ± 130 ng/mL), but again, this difference was not significant.

**Vaccination of mice with rSPEA.** Prior to challenge with H250, these mice had attained a mean weight of 39 g, and there were no significant differences in weight between the groups of animals. Due to the lower-dose inoculum (10⁶ cfu/mouse) and animal size, deaths were not seen until 48 h in this experiment, although the thigh lesion and features of systemic illness during infection were unchanged. The numbers of animals with bacteremia were not significantly different between groups. There were also no differences in the colony counts in animals that were bacteremic. SPEA levels measured during infection were lower in the rSPEA-immune group than in the nonimmune group at each time point, although the difference was not statistically significant (analysis of variance, P = .13). SPEA levels rose to 1.94 ± 1.32 μg/mL (mean ± SD) at 40 h after infection compared with 3.49 ± 2.38 μg/mL in the nonimmune group (figure 6). Of the 12 animals in each group, only 1 from the rSPEA-immunized group survived, compared with 7 survivors in each of the 2 control groups (figure 7), a highly statistically significant difference (P < .001).

**Discussion**

This study allowed detailed analysis of the histologic and systemic responses to lethal group A streptococcal fasciitis in a well-characterized murine model. To our knowledge, we have shown for the first time that SPEA was released systemically during soft-tissue infection with S. pyogenes and that it could...
be concentrated in organs distant from the site of soft-tissue infection. The concentrations of SPEA in sera correlated with IL-6 levels, and both were predictors of reduced survival in this model. This study has also begun to address directly the role of superantigens, such as SPEA, in live sepsis due to S. pyogenes.

The model used in this study attempts to recreate, as much as possible, the clinical illness seen in patients with severe invasive streptococcal disease, although it cannot reproduce all the distinctive features of STSS. In an earlier study, we found that iv injection of H250 into CD1 mice did not cause systemic illness or multiorgan failure but did result in a lethal illness associated with arthritis [13]. Murine models of group A streptococcal sepsis using ip and im injection have been described previously, but there is little information on the pattern of organ damage and inflammatory response [14, 15].

The fasciitis and myonecrosis seen in this model is similar to the histology found in muscle biopsies from our 2 most recent patients with streptococcal necrotizing fasciitis. The mice had histologic evidence of multiorgan failure, with apoptotic cells in the spleen, liver, small bowel, and kidney, suggestive of a toxic effect on tissues rather than bacterial invasion. The changes in spleen, liver, and small bowel were specific to
Serum SPEA in our study was detectable at high levels in infected mice and tended to increase in individual mice before death. To our knowledge, this is the first study to detect and measure circulating SPEA in serum during streptococcal sepsis. Similar levels of H250 SPEA have been measured following overnight culture in enriched medium using this ELISA, but not from strains lacking speA (unpublished data). Western blot analysis of murine sera positive by ELISA for SPEA showed that anti-rSPEA recognized a 26-kDa protein that was not present in controls and that had identity with the 26-kDA H250 native SPEA (data not shown). Methods for measurement of SPEA, reviewed by Alouf et al. [23], have previously used immunodiffusion and ELISA techniques to assay toxin in bacterial broth, but the sensitivity of these assays limits their use. Broth levels of SPEA measured in these earlier studies (1–10 μg/mL) are similar to levels from H250 measured by our ELISA [24, 25].

We are aware of only one previous study that has measured in vivo production of SPEA during infection, though the toxin was present in rabbit tissue cage fluid, formed within an implanted subcutaneous steel net, rather than formed during invasive infection [26]. That study detected 6–9 μg/mL SPEA in tissue cage fluid by radial immunodiffusion, not dissimilar to levels found in our model. Concentrations of toxin in micrograms per milliliter are more than sufficient, in vitro, to cause marked mitogenesis in human mononuclear cell culture and may, in some cases, cause cell death (unpublished data). The current study shows that an in vivo response to superantigen during human streptococcal soft-tissue infection is at least feasible. It is unclear whether such high levels of SPEA would...

Figure 6. Effect of recombinant SPEA (rSPEA) vaccination as shown by SPEA levels during H250 S. pyogenes infection. Error bars show SEs of means of 5 measurements per time point.

Figure 7. Effect of recombinant SPEA (rSPEA) vaccination as shown by Kaplan-Meier survival curve during H250 S. pyogenes infection (n = 12, each group). BSA = bovine serum albumin.
be tolerated in humans, though studies are in progress to establish the extent to which SPEA levels during clinical disease match those measured in the mouse model.

Immunohistochemistry demonstrated specific SPEA staining in the renal tubules and hepatic cells of H250-infected mice but not controls, suggesting that SPEA may be associated with the tubular and hepatic damage seen. Unfortunately, it was not possible, because of the intensity of immunohistochemical staining, to distinguish apoptotic from normal renal tubular cells in these sections. The interaction of SPEA and other bacterial superantigens with cells of nonimmune origin is poorly understood. It is unclear whether SPEA was actively concentrated by the murine kidney or whether it entered tubular cells through cell membrane toxicity. The presence of SPEA in the kidney raised the possibility that impaired renal function may account for the more rapid rise in SPEA in mice that survived <24 h. In a separate experiment, serum creatinine levels, monitored at 0, 5, 15, and 25 h in mice inoculated with an LD50 of H250, did not correlate with SPEA levels (data not shown). It is therefore unlikely that the rise in SPEA was due to impaired renal clearance alone. The localization of SPEA to cells of macrophage origin may reflect the concentration of toxin in immune cells that have ingested phagocytosed bacteria. The staining seen is unlikely to be due to bacteria alone, because no staining was observed in the tissues of the thigh, where streptococcal bacterial colonies were most prevalent.

The elevation of serum IL-6 demonstrates that a systemic inflammatory response was generated in this model. The association of IL-6 with reduced survival in sepsis is well established and is not thought to be causally linked [27]. Of interest, a previous study reported elevated IL-6 levels, but undetectable TNF-α in supernatants from SPEA-stimulated murine splenocytes [21]. In our experiments, the failure to detect serum TNF-α in most animals was surprising but may reflect the more gradual onset of sepsis in this model, compared with existing models of endotoxin-mediated sepsis. It may be that TNF does indeed rise to higher levels at a stage later than when samples were taken, in a pattern similar to IL-6 levels. Notably, however, we have found that neither neutralizing anti-TNF-α nor p55-recombinant soluble TNF receptor can confer protection when given at 0 h, intimating that TNF is unlikely to be a principal mediator of disease in this model (unpublished data). We were intrigued that higher SPEA levels correlated with IL-6 levels and were associated with reduced survival. This raised the possibility that SPEA could be pathogenically important in this model.

To address this issue directly, we first administered rSPEA to CD1 mice but were unable to reproduce the histologic features seen in the H250 sepsis model. This may be because SPEA alone is not responsible for the observed pathology or because rSPEA administration does not reliably mimic SPEA production by H250 during in vivo sepsis. We believe that rSPEA and H250 SPEA are highly similar molecules, on the basis of the genetic sequence of rSPEA (which was derived by PCR from H250), the biologic activity of rSPEA (which is parallel to that of H250 supernatant and commercial SPEA in mitogenesis assays), and the ability of rabbit polyclonal anti-rSPEA to cross-react with SPEA. However, serum rSPEA levels measured after rSPEA administration were detectable only up to 5 h after injection, and it may be that the recombinant protein is in some way degraded in vivo.

To further address the role of SPEA, we evaluated the effect of administering a neutralizing anti-rSPEA antibody to H250-infected animals but a protective effect was not demonstrated. This suggested that SPEA was not a dominant pathogenic factor in this model of fasciitis. Although a SPEA-neutralizing effect would be expected from the dose of antibody administered (two 800-μg doses), we could demonstrate only a small reduction in SPEA levels measured by ELISA in the anti-rSPEA-treated group. This highlights the difficulties encountered when attempting to target neutralizing antibody therapy against a substance being continuously produced in the body. Large and often multiple doses of antibody are required, and it is difficult to conclusively demonstrate neutralization of the target. This may limit the progress of such strategies in the future. The failure of human IVIG to protect the model was of interest, in view of recent anecdotal reports of its successful clinical use in necrotizing fasciitis [28]. A study examining the protective potential of human IVIG as an adjuvant to standard treatment of streptococcal infection is required and may address the issue of whether anti-M, anti- protease, or anti-toxin antibodies are present in different commercial preparations.

The ability to actively immunize against group A streptococcal disease is a desirable goal. It is suggested that patients with low serum antibody levels to SPEA may be at more risk of severe disease due to invasive group A streptococci than are those with higher levels [29]. A previous study reported that immunization of rabbits with SPEA conferred protection against tissue cage infection with SPEA-producing S. pyogenes, though no sham-immunized controls were described, and there was no demonstrable difference between groups in serum responsiveness to SPEA prior to infectious challenge [30]. In that study, bacterial growth and production of SPEA during infection were unaltered in the SPEA-immunized animals, and the mechanism of protection is therefore unclear. We chose to vaccinate mice with purified recombinant SPEA to avoid inadvertent immunization with streptococcal contaminants, such as M protein, cysteine protease, lipoteichoic acid, or hyaluronidase, which may be protective or even harmful in their own right [15, 31–33]. Other than the demonstrable presence of neutralizing anti-rSPEA activity in the rSPEA-immunized mice, there was no other gross difference between the groups tested.

We were surprised to find that mortality was significantly enhanced in the rSPEA-immune group. It is possible that rSPEA given chronically has an unforeseen toxic effect. However, any major effect seems unlikely in view of the maintenance of weight and apparent well-being of this group before
infection. There may have been rSPEA-induced Vβ chain-specific T cell depletion or T cell anergy following the 8-week vaccination course, similar to that seen after chronic administration of staphylococcal enterotoxin B [16]. There is some evidence that SPEA interacts with the Vβ8.2 T cell receptor in the mouse, but this finding is not universal [20, 34]. Alternatively, preformed antibody may predispose to formation of antibody-toxin immune complexes, which could account for the enhanced mortality. The divergence in survival between groups occurred between days 4 and 5, when active immunity to infection would have been developing in all groups. It is possible that vaccination with rSPEA impedes development of active immunity to foreign bacterial antigen, either through induced T cell hyporesponsiveness or through inhibition of immunoglobin synthesis, an in vitro phenomenon attributed to superantigens that has never been properly explained nor observed in vivo [35].

In summary, we have demonstrated, for the first time, the systemic release and localization of a bacterial superantigen (SPEA) in a live sepsis animal model of fasciitis due to S. pyogenes. While levels of SPEA and histologic findings suggest a possible role for SPEA in pathogenicity, both passive and active immunization against SPEA failed to protect animals from death, suggesting that SPEA does not play an important role in invasive disease. Studies aimed at targeted mutagenesis of the speA gene are underway. Intriguingly, mortality was enhanced following vaccination with SPEA, suggesting that superantigen (SPEA)-induced immune dysfunction may reduce resistance to infection.

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References